J Wood Sci (2007) 53:264–267
DOI 10.1007/s10086-006-0843-y

NOTE

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Transmission of mitochondrial plasmids in protoplast cell fusion between compatible monokaryons of *Lentinula edodes*

Received: May 19, 2006 / Accepted: August 23, 2006 / Published online: December 18, 2006

Abstract Electrophoretic analysis of the transmission pattern of mitochondrial plasmids in protoplast cell fusion between compatible monokaryons of *Lentinula edodes* indicates that three of the four plasmids carried in parental monokaryons are effectively transferred and replicated in the protoplast fusants. The two monokaryons, 1158a and 1569a, carried different plasmids that could be distinguished by a single restriction digest. Electrophoresis of intact plasmids and restriction analyses indicate that all but one of the fusants carry three of the four possible plasmids, indicating that transmission of plasmids in protoplast fusions is principally biparental in *L. edodes*. Thus, heterocytoplasmic cells of *L. edodes* can be effectively constructed by protoplast cell fusion. In addition, plasmids of the same homology group cannot coexist in the heteroplasmic cells after protoplast cell fusion.

Key words Mitochondrial plasmid · Protoplast cell fusion · *Lentinula edodes*

Introduction

Shiitake, *Lentinula edodes* (Berk.) Pegler, is a commercially important edible mushroom cultivated in many countries, particularly in Japan and China. Although numerous cultivars are available, additional cultivars are necessary to meet changing agronomic and economic requirements. Intraspecific protoplast cell fusion between compatible monokaryons has made possible the development of new strains carrying useful properties such as high fruiting body productivity in *L. edodes*.[1] To take full advantage of protoplast cell fusion in *L. edodes* breeding, it would be beneficial to clarify the genetic differences between protoplast fusants and strains constructed by sexual mating using the same compatible monokaryons.

Fukuda et al.[2] studied transmission patterns of mitochondrial genome DNA (mtDNA) in protoplast cell fusion of *L. edodes* and found possible recombinant mtDNA in some protoplast fusants. However, although Fukumasa-Nakai et al.[3] characterized six different linear mitochondrial plasmids and demonstrated that their transmission pattern in sexual crosses is uniparental, there is no evidence for behavior of the plasmids in protoplast cell fusion. In this study we investigated the transmission of mitochondrial plasmids in protoplast cell fusants between compatible monokaryons of *L. edodes*.

Materials and methods

Fungal strains

Two compatible monokaryotic strains of *Lentinula edodes*, 1158a and 1569a, and 13 electrofusion protoplast fusants (F1–F13) between 1158a and 1569a produced in a previous study[2] were used. Strains 1158a and 1569a were produced through artificial dedikaryotization by the protoplast regeneration method[4] from two wild dikaryotic strains, TMIC-1158 from Japan and TMIC-1569 from New Zealand, which were deposited in the culture collection of the Tottori Mycological Institute.

Isolates F1–F13 were assumed to be protoplast fusants because no colonies with clamp connections developed in control tests in which the fusion treatment was omitted, as described previously.[2]

DNA isolation and digestion

To prepare mycelium for total DNA extraction, cultures were grown without agitation in MYG (2% malt extract,

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Contribution No. 382 of the Tottori Mycological Institute

0.2% yeast extract, 2% glucose) liquid medium at 25°C for 14 days and fragmented with a Waring blender; 10 ml was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml MYG liquid medium. The flask cultures were incubated in a stationary state in darkness at 25°C for 14 days, harvested, washed with distilled water, and lyophilized.

Extraction of total DNA from lyophilized mycelia was achieved by following the procedure of Fukumasa-Nakai et al.[3] A portion of the total DNA was examined by electrophoresis on a 1% agarose (Type S, Nippon Gene, Tokyo, Japan) slab gel in TAE [40 mM Tris/acetate, 10 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0] at 5 V/cm for 4 h for plasmid detection. Plasmids were detected on an ultraviolet (UV) transilluminator after staining the gel with ethidium bromide (0.5 μg/ml). Plasmids were removed from the gel and purified using a QIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany) following the supplier's instructions and digested with *Bam*HI (Nippon Gene). Plasmid digests were electrophoresed as above. Lambda phage DNA digested with *Hin*dIII was used as a molecular size standard.

Results and discussion

In electrophoretic analysis, both parental monokaryons of *Lentinula edodes* used in this study were found to retain a set of all plasmids harbored by their original dikaryons, namely two plasmids [11.1 kb (pLE2) and 12.1 kb (pLE3C)] for 1158a and its derived dikaryon TMIC-1158, and two plasmids [9.0 kb (pLE1) and 12.3 kb (pLE3D)] for 1569a and its derived dikaryon TMIC-1569, as reported previously[3] (Fig. 1).

Fusants resulting from the two monokaryons have the theoretical possibility of containing all four of the mitochondrial plasmids. However, it was found that almost all of the 13 fusants (F2–F13) examined in the present study carried three plasmid bands, with the exception of fusant F1 having two plasmid bands (Fig. 2). To determine whether such plasmid bands of fusants conform to any plasmids of the parental monokaryons, a course of restriction analyses was performed. Because electrophoresis could not sufficiently resolve the high molecular weight plasmids for excising them individually, they were removed together from the gels and used for the present restriction analysis. The two plasmids (pLE2 and pLE3C) from 1158a produced *Bam*HI fragments of 7.3, 6.5, 4.6, 3.0, and 2.1 kb (Fig. 3). Among these bands, the 6.5- and 4.6-kb bands were in accordance with those from pLE2, and the remaining three bands from pLE3C, as reported by Fukumasa-Nakai et al.[3] The *Bam*HI digestion pattern of pLE3D from 1569a, which consists of three restriction bands of 5.8, 3.8, and 2.7 kb (Fig. 3), was identical to published results.[3] Thus, pLE3C and pLE3D could be distinguished from each other by *Bam*HI digestion. *Bam*HI restriction pattern of the two high molecular weight bands from each of the 13 fusants matched the pattern of 1158a plasmids (Fig. 4), indicating that the fusants carry the two 1158a plasmids. In addition, because there is no *Bam*HI restriction site in pLE1,[3] the plasmid recovered from 1569a should produce no *Bam*HI restriction fragments. The 9.0-kb bands from the 12 fusants also produced no restriction band (data not shown). This indicates

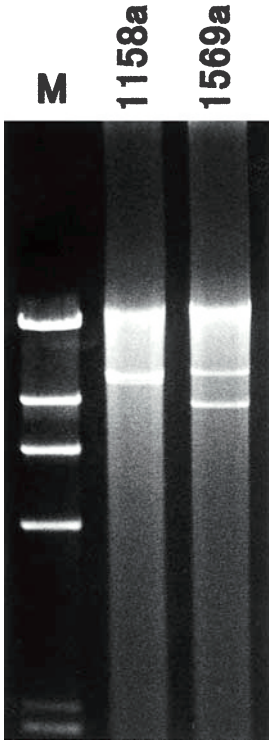

Fig. 1. Electrophoretic analysis of linear plasmids from 1158a (pLE2: 11.1 kb and pLE3C: 12.1 kb) and 1569a (pLE1: 9.0 kb and pLE3D: 12.3 kb). Lane *M*, *Hin*dIII-digested lambda phage DNA

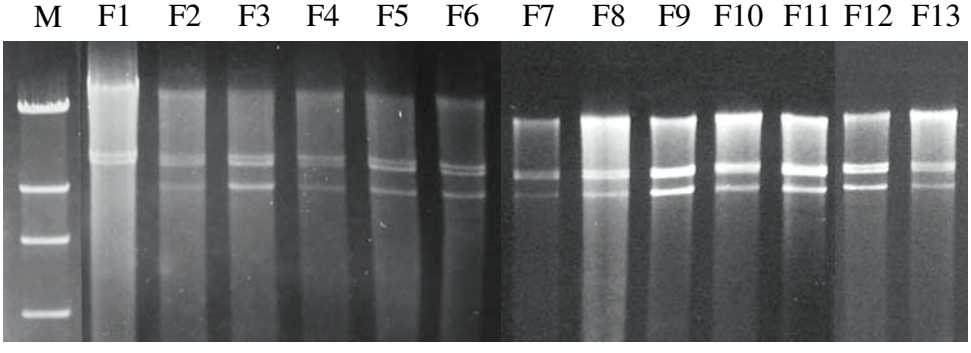

Fig. 2. Electrophoretic analysis of plasmids from 13 protoplast fusants of strains 1158a and 1569a of *Lentinula edodes*. Lane *M*, *Hin*dIII-digested lambda phage DNA

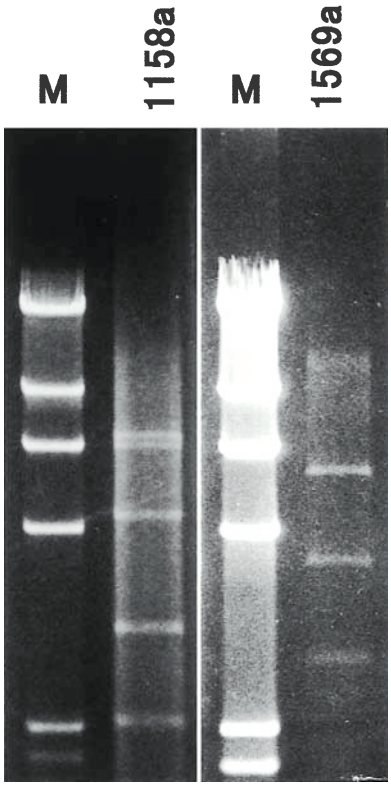

Fig. 3. *Bam*HI restriction patterns of plasmids from 1158a and 1569a. *Bam*HI digestion results in fragments of 7.3, 6.5, 4.6, 3.0, and 2.1 kb from pLE2 and pLE3C, and fragments of 5.8, 3.8, and 2.7 kb from pLE3D. Lane *M*, *Hin*dIII-digested lambda phage DNA

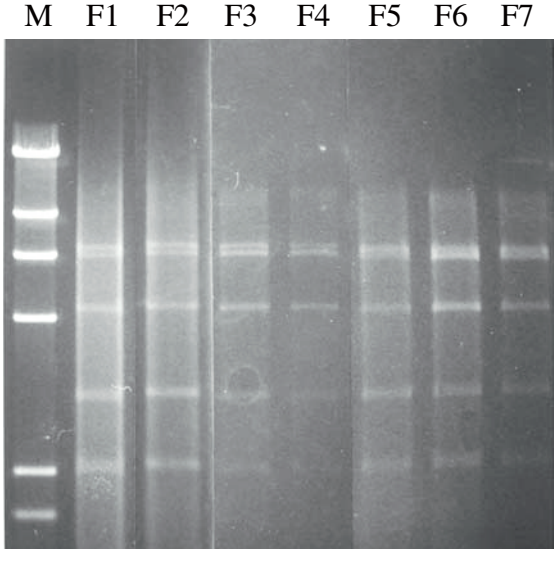

Fig. 4. *Bam*HI restriction patterns of the two high molecular weight plasmids from nine representative protoplast fusants. Lane *M*, *Hin*dIII-digested lambda phage DNA

that the 9.0-kb band detected in the 12 fusants was pLE1 from 1569a. Therefore, these results indicate that the protoplast fusants carry pLE2 (11.1 kb) and pLE3C (12.1 kb) from 1158a and fusants F2–F13 also carry pLE1 (9.0 kb) from 1569a.

The cause for uniparental inheritance of plasmids in fusant F1 is not clear. On the fusant, deficiency of the 1569a plasmids possibly occurs during the cell division after protoplast cell fusion. On the other hand, the contribution of cytoplasmic components from the parental monokaryons may not be equal or constant during electrofusion. There is thus the possibility that fusant F1 resulted from a fusion between a protoplast with the intracellular composition of 1158a and a karyoplast of 1569a. In addition, fusant F1 carries the mtDNA genotype from 1158a.[2]

Protoplast fusion can thus result in plasmid contributions from both parent strains. In the case of 1158a and 1569a, both strains are capable of donating plasmids. The apparent lack of transfer or replication of pLE3D may be due to incompatibility with one of the 1158a plasmids, but cannot be explained by this study. Plasmids pLE3C and pLE3D are in the same homology group and have not been detected concurrently in any wild strain of *L. edodes*.[3] Plasmid pLE3C is clearly transmitted and effectively replicated in fused protoplasts, but the mechanisms of plasmid selection in heteroplasmic fungal cells are not clear. Hereafter, further studies are required to clarify the causes of such phenomena.

Among 90 wild strains of *L. edodes*, about one third carry two or three different plasmids.[3] However, the combination of pLE1, pLE2, and pLE3C in the 12 fusants is unique because pLE1 is found only in Papua New Guinea and New Zealand and pLE3C is found only in Japan.[3] Because uniparental transmission of the mitochondrial plasmids has been reported in *L. edodes* sexual crosses,[3] intraspecific protoplast cell fusion could be a useful method for increasing the cytoplasmic variability of *L. edodes*.

The mtDNA genotypes of the fusants used in this study have been analyzed: biparental transmission of mitochondria was observed for fusants F10–F13, and the remaining nine fusants carry one or the other of two mtDNA genotypes from the parental monokaryons.[2] Thus, biparental transmission of mitochondria has been observed only in the four fusants, but biparental transmission of plasmids was evident in all but one of the fusants. The results of the present study indicate that heterocytoplasmic cells may be formed frequently by protoplast cell fusion. In contrast, uniparental plasmid transmission has been demonstrated in sexual crosses of *L. edodes*.[3] In the case of sexual crosses of this fungus, there may be little opportunity to detect the heterocytoplasmic cells. This is because only a few cytoplasmic organelles including mitochondria may migrate from the donor cell to the recipient through the opening formed by hyphal anastomoses between the paired monokaryons. It is probable that the dikaryotization and later cell division occurs immediately after the hyphal anastomoses without mitochondrial transmission from the donor monokaryon, judging from the microscopic observation reported by Nakai.[5]

The present study revealed that biparental plasmid transmission occurs between compatible monokaryons of *L. edodes* in protoplast cell fusion. It may be useful to increase cytoplasmic variability for *L. edodes* breeding because extranuclear DNA, including plasmids, may affect phenotypic properties of *L. edodes*, as recognized for mycelial growth and isozyme patterns in two reciprocal dikaryons with the same nuclei, but with different mitochondrial types.[1] To clarify the possible influences of mitochondrial plasmids on phenotypic properties, and to reveal the utility of protoplast cell fusion for *L. edodes* breeding, it will be important to determine the specific agronomic characters of each fusant. In addition, the stability of the extranuclear DNA of the protoplast fusants will have to be studied.

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